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A New Taxane Diterpenoid from the Seeds of *Taxus yunnanensis*

Qing Wen SHI[1], Takayuki ORITANI[1], Ding ZHAO[2]

[1]Laboratory of Applied Bioorganic Chemistry, Division of Life Science, Graduate School of Agricultural Sciences, Tohoku University,
Tsutsumidori-Amamiya, Aoba-ku, Sendai 981-8555, Japan
[2]Department of Chemistry of Medicinal Natural Products, Faculty of Pharmaceutical Sciences, Hebei Medical University, 361 Zhongshan East Street, Shijiazhuang 050017

Abstract: A new taxane diterpenoid with a rearranged 5/7/6-membered ring system was isolated from the seeds of the Chinese yew, *Taxus yunnanensis*. Its structure was established as 9α-acetoxy-5α-cinnamoyl-11(15 → 1)-abeotaxa-4(20), 11-diene-10β, 13α, 15-triol on the basis of spectroscopic analysis. Its relative stereochemistry is proposed from the results of a NOESY experment.

Keywords: *Taxus yunnanensis*, taxoid, isolation, *Taxus*.

Plant from the genus of *Taxus* are a rich source of biologically active diterpennods belonging to the unique structural family of taxoids[1]. Although more than 300 taxoids were isolated from yew tree[2], new taxoids still were isolated. In our continuing studies on the yew tree, we have previously isolated several new taxoids from seeds of the Chinese yew, *Taxus chinensis var.* mairei. *T. yunnanensis*, and Japanese yew, *T. Cuspidata*[3-5]. Further investigation on the extract of the seeds of *T. yunnanensis* resulted in the isolation of a new taxoid **1**. In this communication we describe the isolation and the structure elucidation of this new compound.

The crushed seeds (2.2kg) of *T. yunnanensis* were de-fatted and extracted with methanol. The methanolic extract was treated with activated charcoal and subjected to silica gel column chromatography followed by preparative TLC to afford compound **1** in a 0.00024% yield based on the dry material as a colorless gummy substance. The molecular formula of compound **1** was established to be $C_{31}H_{40}O_7$ by combined analysis of the HRFAB-MS and ^{13}C-NMR spectrum. The extensive absorptions at 3400, 1730, 1700 and 1630 cm^{-1} in the IR spectrum implied that **1** possessed hydroxyl, ester, α,β-unsaturated ester groups and α,β-unsaturated double bond, respectively. The ^{1}H-NMR spectrum of **1**, tabulated in **Table 1**, exhibited the proton signals due to the four methyl group at δ 0.78, 1.09, 1.36, and 1.93, one acetyl group resonated at δ2.12, which was confirmed by the observation of ^{13}C-NMR signals at δ172.4 and 20.0. These signals suggested that **1** had a taxane-type skeleton. Proton signals due to a cinnamoyl group were observed at δ7.52 (2H, m), 7.38 (3H, m), 7.64 (1H, d, J=16.0Hz), and 6.44 (1H, d, J=16.0Hz; *trans*-oriented). UV absorption at 279 nm and prominent fragment

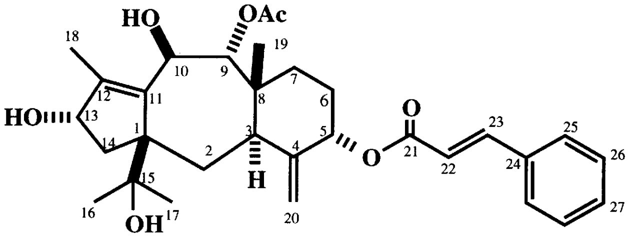
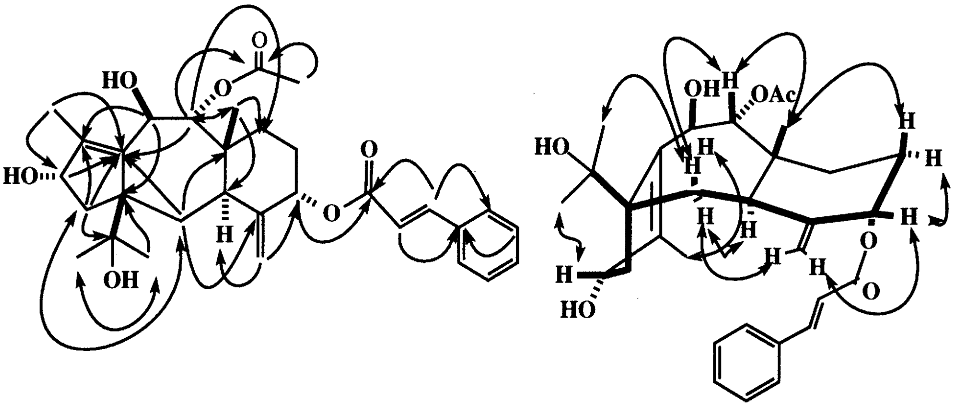


Table 1. ^{1}H-(300MHz) and ^{13}C-NMR(125MHz) spectral data for **1** in CDCl$_3$

Position	**^{1}H**	**J**	**^{1}H-^{1}H COSY**	**^{13}C**
1				62.5
2α	1.38 d	14.0	H-2β	30.4
2β	2.00 dd	14.0, 8.0	H-3, 2α	
3	2.85 d	8.0	H-2α	41.5
4				148.3
5	5.45 brs		H-6	76.1
6	1.92 m		H-5, 7	28.5
7	1.74 m		H-6	29.8
8				42.3
9	5.53 d	10.0	H-10	81.8
10	4.50 d	10.0	H-9	78.3
11				135.2
12				140.1
13	4.5 t	7.2	H-14α, 14β, 18	68.7
14α	1.10 d	13.7	H-13, 14β	48.2
14β	2.30 dd	13.7, 7.2	H-13, 14α	
15				78.3
16	1.36 s			26.6
17	1.09 s			28.2
18	1.93 d	0.8	H-13	11.9
19	0.78 s			17.8
20a	5.20 brs		H-20b	112.7
20b	4.78 brs		H-20a	
21				166.6
22	6.44 d	16.0	H-23	119.4
23	7.64 d	16.0	H-22	145.4
24				130.5
25	7.52 m		H-26	128.8
26	7.38 m		H-25	129.6
27	7.38 m			131.0
9-AcO	2.12 s			20.0
				172.4

peak at *m/z* 131 (C_9H_7O) in FAB-MS supported the presence of a cinnamoyl group in **1**. The connectivities of the protons at the taxane skeleton of **1** were determined by an analysis of the ^{1}H-^{1}H COSY spectrum. Interpretation of ^{1}H-, ^{13}C-NMR, HMQC and HMBC spectra permitted the positional assignment of functional groups. The ^{1}H-NMR signals at δ5.20 (1H, brs), 4.78 (1H, brs) and 2.85 (1H, d, J=8.0Hz) are characteristic of an exocyclic methylene and C-3 ring junction proton in a taxa-4(20), 11-diene, respectively[6]. Additionally, four oxymethine protons appeared at lower field, and were assigned as H-5β, H-9β, H-10α, and H-13β on the basis of ^{1}H-^{1}H COSY and HMBC spectra. Large vicinal coupling indicated a *trans*-oriented configuration of the H-9β and H-10α. The spin system derived from 18-CH$_3$, H-13β, H-14α, H-14β was readily interpreted. The C-11 and C-12 carbon signals showed cross-peaks with the H-14β resonance, suggesting that both C-11 and C-12 are three bonds apart from H-14β. This means that the A ring was a cyclopentene as in an 11(15→1)-*abeo*taxane structure[7]. The carbon signal at δ78.3, assigned to hydroxyl-bearing C-15, displayed a cross-peak with the C-16 and C-17 methyl resonances at δ1.36 and 1.09. The C-1 signal (δ62.5), apart from from H-10α, also showed three-bond coupling with the H-3α and C-16 , C-17 methyl signals. Since no cross-peak was observed between C-16, C-17 (methyl) signals and C-11 olefinic carbon in the HMBC spectrum further supported the 11(15→1)-*abeo*taxane skeleton for **1**. The locations of an acetyl group and a cinnamoyl group were deduced at C-9 and C-5 from the HMBC spectrum (**Figure 1**). The relative stereochemistry of the terpenoid skeleton of **1** was determined from the chemical shifts, coupling constants and NOESY experiment. A coupling constant between H-9 and H-10 of *J*=10.0 Hz indicated that the B-ring was the chair-boat conformation. The NOESY experiment established the relative stereochemistry of **1** at all positions, and the results are shown in **Figure 1**. Thus the structure of **1** was established as 9α-acetoxy-5α-cinnamoyl-11(15→1)-*abeo*taxa-4(20), 11-diene-10β, 13α, 15-triol.

Figure 1. HMBC Correlations used in establishing the abeotaxane skeleton (left, most protons are omitted for clarity) and relative stereochemistry of **1** proposed on NOESY experiment (500 MHz).



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